Docket No.21772-3A

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Ryan O. White

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Applicant:	Charles Thivolet	:	
Serial No.:	To be assigned	:	Group Art Unit:
Filing Date:	January 16, 2002	:	Examiner:

For: **Use of IGF-I or Analogues Thereof in the Prevention of Diabetes**

PRELIMINARY AMENDMENT

Box Patent Application
U.S. Patent and Trademark Office
Washington, DC 20231

Dear Sir:

Prior to calculation of the filing fee and first action by the Examiner, please amend the present application as follows:

In the Drawings:

Please delete Figure 3.

In the Specification:

After the Title of the Invention on page 1, please insert the following paragraph:

--This a continuation of Application Serial No. 08/750,733 filed on April 7, 1997.--

Please rewrite the paragraph appearing at page 2, lines 15-26 to read as follows:

--Insulin like growth factor-1 (IGF-1), a 70-amino acid peptide structurally related to insulin, is normally considered to be a metabolic hormone which mediates many effects of growth hormone. Prophylactic insulin treatment of NOD mice during the prediabetic phase (5) as well as insulin treatment of the NOD recipients of autoreactive T cells during adult T cell transfer (6) have been shown to prevent and/or delay the onset of diabetes and to reduce the severity of insulitis. Similar results have been also obtained in BB rats (7,8), which are another animal model of spontaneous autoimmune diabetes. Since insulin is a major antigenic component of the beta cells, it was not clear from these experiments whether insulin protective effects were explained by an antigen-specific unresponsiveness of the immune system, by a direct suppressive effect on T cell function, or by a direct effect on the beta cells.--

Please rewrite the paragraph bridging pages 2 and 3 to read as follows:

--IGF-I has also shown to have a protective effect against diabetes, in preventing beta cell destruction in subjects which are at high risk of development of diabetes and in the regulation of T cells in subjects which are at high risk of developing diabetes.--

Please rewrite the paragraphs referencing the Figures and appearing at page 3, lines 10-20 to read as follows:

-- The following figures are illustrating the results of the experiments.

Figure 1 Cumulative incidence of diabetes in four independent experiments in mice;

Figure 2 Severity of insulitis and destructive lesions;

Figures 3a-3c FACS analysis of Thy-1,2$^+$ T cells within the spleen of a congenic NOD-N Thy-1,1 mouse;

Figures 4a-4c FACS analysis of Thy-1,2$^+$ T cells within the thymus of a congenic

NOD-N Thy-1,1 mouse.--

Please rewrite the paragraph appearing at page 7, lines 5-16 to read as follows:

--Because insulitis is a T cell phenomenon, we suspected that rhlGF-1 might interfere with the kinetics of the migration of committed T cells to the pancreas. Congenic NOD-N Thy-1,1 males were adoptively transferred with T cells from diabetic NOD Thy-1,2 animals. Diabetes occurred in 3 / 6 mice that had been treated with saline and 0 / 6 mice that had been treated with rhlGF-1, after 3 weeks of treatment. This apparent protective effect was also associated with a decrease in the severity of islet cell infiltrates, which were composed exclusively by T cells from donor origin with no recruitment of host T cells. More particularly, immunodetection of Thy-1,2^{+} T cells in the islets of congenic NOD-N Thy-1,1 mice three weeks after adoptive cell transfer of diabetes using $7x10^{6}$ T cells from NOD Thy-1,2 diabetic donors illustrates a severe insulitis in a control mouse while illustrating a peri-insulitis in a mouse treated with rhlGF-1. Additionally, when analyzed in individual mice, the number of Thy-1,2^{+} T cells was found to be significantly lower in the spleen of treated mice with rhlGF-1 in comparison with control mice (Table III and Figures 3a, 3b and 3c), although no significant difference was noted within the thymus (Figures 4a, 4b and 4c).--

Please rewrite the paragraph appearing at page 8, lines 12-32 to read as follows:

--The observation of pancreatic glands free from insulitis under rhlGF-1 treatment, suggests another mechanism that occurs prior to the late activation process of infiltrating T cells by eliminating or inactivating the functional properties of autoreactive T cells necessary for beta cell destruction. Recombinant hlGF-1 may exert these effects directly on lymphoid cells, since in vitro suppression of T cell response to concanavalin A or allogeneic stimulation can be achieved in a dose dependent manner (16). Many actions of growth-

hormone on the immune system may be mediated by IGF-1 which is also produced by peripheral leukocytes (17). Recent observations suggest that activated T lymphocytes possess receptors for IGF-1 (18-20). In addition, several reports indicate that IGF-1 may influence thymic epithelial cell function in vitro (21) and induce thymocyte replication and differentiation in streptozocin induced diabetic rats (22). Mice which receive 4 mg/kg per day of rhlGF-1 were found to have an increased spleen and thymus weight, due to an increase in the number of lymphocytes in these organs, preferentially T cells from the CD4 phenotype (22). We did not observe any difference in the number of T cells in the lymphoid organs and in the relative contribution of T cell subsets within the spleen, probably because of lower doses of rhlGF-1 used in the present study. Moreover, treatment of diabetic females with rhlGF-1 failed to reduce the capacity of spleen cells to transfer the disease, suggesting that the number and degree of activation of autoreactive T cells were not modified.--

Please rewrite the paragraphs referencing the LEGENDS OF FIGURES and appearing at page 9, lines 12-37 to read as follows:

--LEGENDS OF FIGURES

Figure 1: Cumulative incidence of diabetes in four independent experiments following adoptive T cell transfer in 24 mice injected twice daily with 10μ rhlGF-1 (open circles) and 21 control mice injected with saline (closed circles).

Figure 2: Severity of insulitis and destructive lesions of recipient mice according to treatment with saline (dark columns) or rhlGF-1 (open columns). Results are mean percentages ± SE from 24 individual mice from two independent experiments.

Figures 3a, 3b and 3c: FACS analysis of Thy-1,2$^+$ T cells within the spleen of a congenic NOD-N Thy-1,1 mouse, three weeks after sub-lethal irradiation and inoculation of Thy-1,2$^+$ T cells from diabetic donors. Figure 3a represents the results

in a control NOD-N Thy-1,1 mouse. Insulin like growth factor-1 significantly reduced the number of Thy-1,2^+ in the spleen (Figure 3b) in comparison to saline (Figure 3c).

Figures 4a, 4b and 4c: FACS analysis of Thy-1,2^+ T cells within the thymus of a congenic NOD-N Thy-1,1 mouse, three weeks after sub-lethal irradiation and inoculation of Thy-1,2^+ T cells from diabetic donors. Figure 4a represents the results in a control NOD-N Thy-1,1 mouse. The effects of rhIGF-1 upon the reconstitution of the thymus after T cell transfer are shown in Figure 4b and are compared to saline injected mouse (Figure 4c).--

In the Claims:

Please cancel claims 1-6 and 8

Please amend claims 7, 9 and 10 to read as follows:

7. (Amended) Method for delaying the clinical onset of diabetes by administration of an IGF-I analogue.

9. (Amended) Method for reducing the occurrence of beta cell destruction in a subject having a high risk of developing diabetes by administration of an IGF-I analogue.

10. (Amended) Method for reducing the number of T cells migrating to the spleen in a subject having a high risk of developing diabetes by administration of an IGF-I analogue.

Please add the following claims 11-27:

--11. (NEW) A method according to claim 7, wherein the IGF-I analogue is administered in a daily dosage of from 20 to 500 µg/kg.--

--12. (NEW) A method according to claim 9, wherein the IGF-I analogue is administered in a daily dosage of from 20 to 500 μg/kg.--

--13. (NEW) A method according to claim 10, wherein the IGF-I analogue is administered in a daily dosage of from 20 to 500 μg/kg.--

--14. (NEW) A method according to claim 7, wherein the IGF-I analogue is administered in a daily dosage of from 20 to 250 μg/kg.--

--15. (NEW) A method according to claim 9, wherein the IGF-I analogue is administered in a daily dosage of from 20 to 250 μg/kg.--

--16. (NEW) A method according to claim 10, wherein the IGF-I analogue is administered in a daily dosage of from 20 to 250 μg/kg.--

--17. (NEW) A method according to claim 7, wherein the IGF-I analogue is administered in a daily dosage of from 100 to 200 μg/kg.--

--18. (NEW) A method according to claim 9, wherein the IGF-I analogue is administered in a daily dosage of from 100 to 200 μg/kg.--

--19. (NEW) A method according to claim 10, wherein the IGF-I analogue is administered in a daily dosage of from 100 to 200 μg/kg.--

--20. (NEW) A method according to claim 7, wherein the IGF-I analogue has a sequence identity of at least 90% to IGF-I.--

--21. (NEW) A method according to claim 9, wherein the IGF-I analogue has a sequence identity of at least 90% to IGF-I.--

--22. (NEW) A method according to claim 10, wherein the IGF-I analogue has a sequence identity of at least 90% to IGF-I.--

--23. (NEW) A method according to claim 7, wherein the IGF-I analogue has a sequence identity of at least 95% to IGF-I.--

--24. (NEW) A method according to claim 9, wherein the IGF-I analogue has a sequence identity of at least 95% to IGF-I.--

--25. (NEW) A method according to claim 10, wherein the IGF-I analogue has a sequence identity of at least 95% to IGF-I.--

--26. (NEW) A method according to claim 7, wherein the IGF-I analogue has a sequence identity of at least 99% to IGF-I.--

--27. (NEW) A method according to claim 9, wherein the IGF-I analogue has a sequence identity of at least 99% to IGF-I.--

REMARKS

By the present amendment, the specification is amended to correct various matters of form on pages 2, 3 and 8. Additionally, Applicant has canceled Figure 3 and renumbered original Figures 4 and 5 as well as updated the specification accordingly. Support for Figures 3a, 3b and 3c may be found in original Figure 4, while support for Figures 4a, 4b and 4c may be found in original Figure 5. Support for adding the sentence regarding immunodetection of Thy-1,2^{+} T cells in the islets of congenic NOD-N Thy-1,1 mice three weeks after adoptive cell transfer to the paragraph appearing at page 7, lines 5-16, may be found at page 9, lines 21-25 of the original specification as filed. Additionally, claims 7, 9 and 10 are amended to recite IGF-I analogues and claims 1-6 and 8 are cancelled. Furthermore, claims 11-19 are added to more specifically recite the dosage requirements of the IGF-I analogues, while claims 20-27 are added to more specifically recite the sequence identity of the IGF-I analogues. A Version With Markings Showing Changes Made is attached. It is believed that these changes do not involve any introduction of new matter, whereby entry is believed to be in order and is respectfully requested.

Respectfully submitted,

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Dinsmore & Shohl LLP
1900 Chemed Center
255 East Fifth Street
Cincinnati, Ohio 45202
(513) 977-8686

752010.01

VERSION WITH MARKING SHOWING CHANGES MADE

In the Specification:

The paragraph appearing at page 2, lines 15-26 is amended as follows:

--Insulin like growth factor-1 (IGF-1), a 70-amino acid peptide structurally related to insulin, is normally considered to be a metabolic hormone which mediates many effects of growth hormone. Prophylactic insulin treatment of NOD mice during the prediabetic phase (5) as well as insulin treatment of the NOD recipients of autoreactive T cells during adult T cell transfer (6) have been shown to prevent and/or delay the onset of diabetes and to reduce the severity of insulitis. Similar results have been also obtained in BB rats (7,8), which [as] are another animal model of spontaneous autoimmune diabetes. Since insulin is a major antigenic component of the beta cells, it was not clear from these experiments whether insulin protective effects were explained by an antigen-specific unresponsiveness of the immune system, by a direct suppressive effect on T cell function, or by a direct effect on the beta cells.--

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--[5] The following figures are illustrating the results of the experiments.

Figure 1 Cumulative incidence of diabetes in four independent experiments in mice;

Figure 2 Severity of insulitis and destructive lesions;

[Figure 3 Immunodetection of Thy-1,2$^+$ T cells in the islets of congenic NOD-N Thy-1,1 mouse]

[Figure 4] Figures 3a-3c FACS analysis of Thy-1,2$^+$ T cells within the spleen of a congenic NOD-N Thy-1,1 mouse; and

[Figure 5] Figures 4a-4c FACS analysis of Thy-1,2$^+$ T cells within the thymus of a congenic NOD-N Thy-1,1 mouse.--

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The paragraph appearing at page 8, lines 12-32 is amended as follows:

--The observation of pancreatic glands free from insulitis under rhlGF-1 treatment, suggests another mechanism that occurs prior to the late activation process of infiltrating T cells by eliminating or inactivating the functional properties of autoreactive T cells necessary for beta cell destruction. Recombinant hlGF-1 may exert these effects directly on lymphoid cells, since in vitro suppression of T cell response to concanavalin A or allogeneic stimulation can be achieved in a dose dependent manner (16). Many actions of growth-hormone on the immune system may be mediated by IGF-1 which is also produced by peripheral leukocytes (17). Recent observations suggest that activated T lymphocytes possess receptors for IGF-1 (18-20). In addition, several reports indicate that IGF-1 may influence thymic epithelial cell function in vitro (21) and induce thymocyte replication and differentiation in streptozocin induced diabetic rats (22). Mice which receive 4 mg/kg per day of rhlGF-1 were found to have an increased spleen and thymus weight, due to an increase in the number of lymphocytes in these organs, preferentially T cells from the CD4 phenotype (22). We did not observe any difference in the number of T cells in the lymphoid organs and in the relative contribution of T cell subsets within the spleen, probably because of lower doses of rhlGF-1 used in the present study. Moreover, treatment of diabetic females with rhlGF-1 failed to reduce the capacity of spleen cells to transfer the disease, suggesting that the number and degree of activation of autoreactive T cells were not modified.--

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Figure 2: Severity of insulitis and destructive lesions of recipient mice according to treatment with saline (dark columns) or rhlGF-1 (open columns). Results are mean percentages ± SE from 24 individual mice from two independent experiments.

[Figure 3: Immunodetection of Thy-1,2$^+$ T cells in the islets of congenic NOD-N Thy-1,1 mice three weeks after adoptive cell transfer of diabetes using $7x10^6$ T cells from NOD Thy-1,2 diabetic donors. Panel A illustrates a severe insulitis in a control mouse. Panel B represents a peri-insulitis in a mouse treated with rhlGF-1.]

[Figure 4] Figures 3a, 3b and 3c: FACS analysis of Thy-1,2$^+$ T cells within the spleen of a congenic NOD-N Thy-1,1 mouse, three weeks after sub-lethal irradiation and inoculation of Thy-1,2$^+$ T cells from diabetic donors. [Panel A] Figure 3a represents the results in a control NOD-N Thy-1,1 mouse. Insulin like growth factor-1 significantly reduced the number of Thy-1,2$^+$ in the spleen [(Panel B)] (Figure 3b) in comparison to saline [(Panel C)] (Figure 3c).

[Figure 5] Figures 4a, 4b and 4c: FACS analysis of Thy-1,2$^+$ T cells within the thymus of a congenic NOD-N Thy-1,1 mouse, three weeks after sub-lethal irradiation and inoculation of Thy-1,2$^+$ T cells from diabetic donors. [Panel A] Figure 4a represents the results in a control NOD-N Thy-1,1 mouse. The effects of rhlGF-1 upon the reconstitution of the thymus after T cell transfer are shown in [Panel B] Figure 4b and are compared to saline injected mouse [(Panel C)] (Figure 4c).--

In the Claims:

Claims 7, 9 and 10 are amended as follows:

7. (Amended) Method for delaying the clinical onset of diabetes by administration of an IGF-I [or analogues thereof] analogue.

9. (Amended) Method for [preventing] reducing the occurrence of beta cell destruction in [subjects which are at] a subject having a high risk of developing diabetes by administration of an IGF-I [or analogues thereof] analogue.

10. (Amended) Method for [regulating] reducing the number of T cells migrating to the spleen in [subjects which are at] a subject having a high risk of developing diabetes by administration of an IGF-I [or analogues thereof] analogue.

Title: Use of IGF-I or Analogues Thereof in
the Prevention of Diabetes
Applicant: Thivolet, Charles
Serial No.: TO BE ASSIGNED
Docket: 21772-3

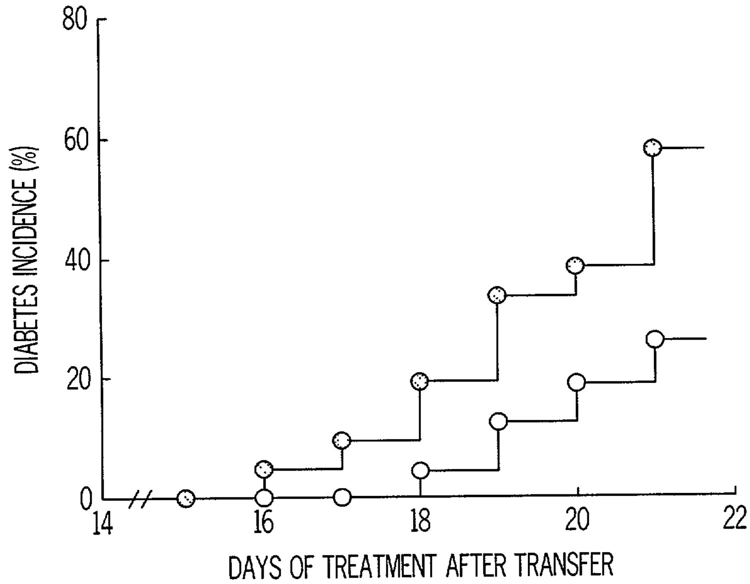

FIG. 1

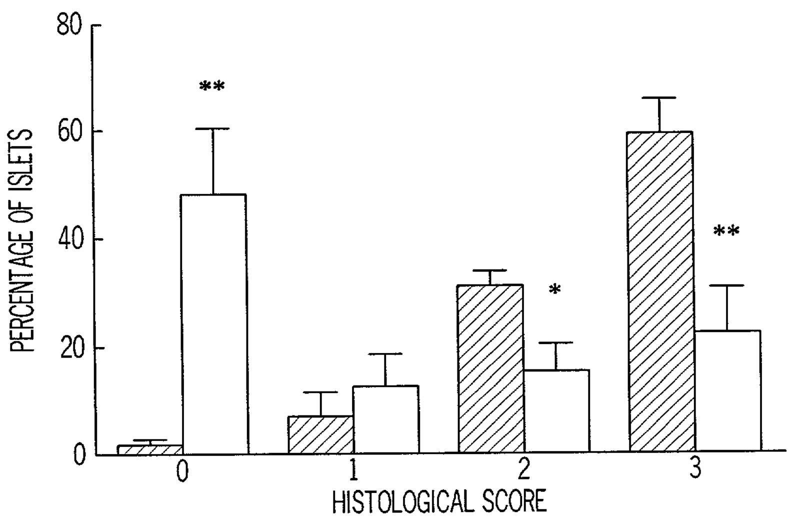

FIG. 2

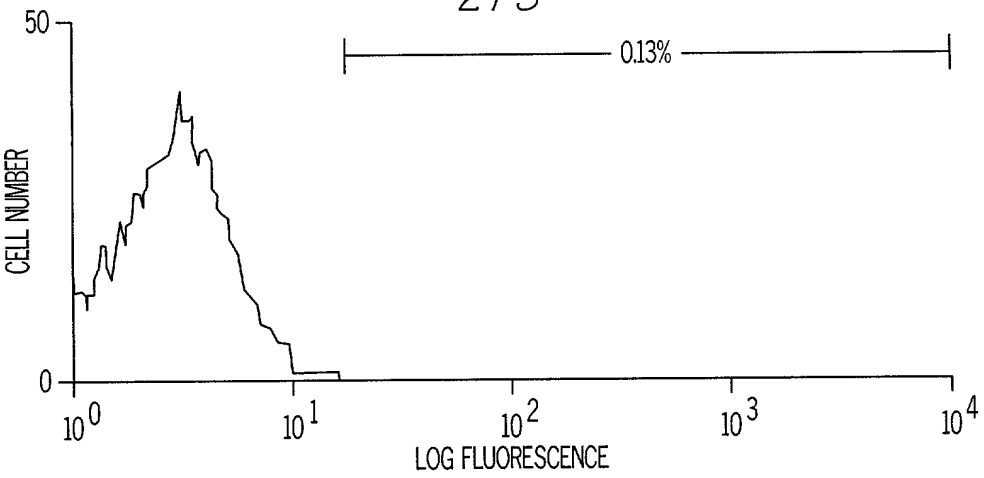

FIG. 3A

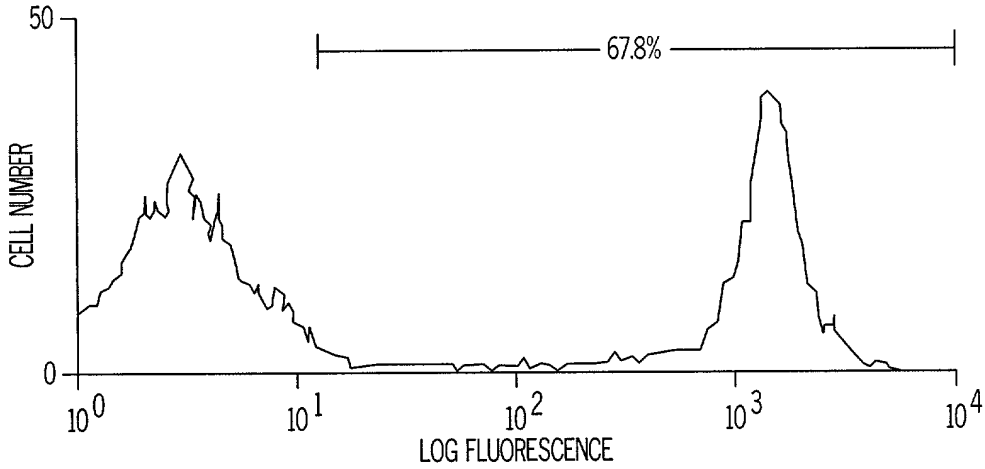

FIG. 3B

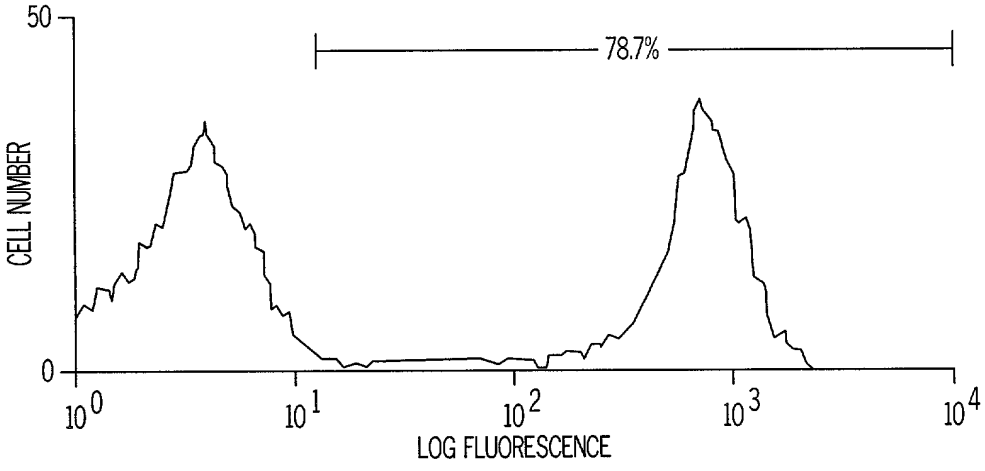

FIG. 3C

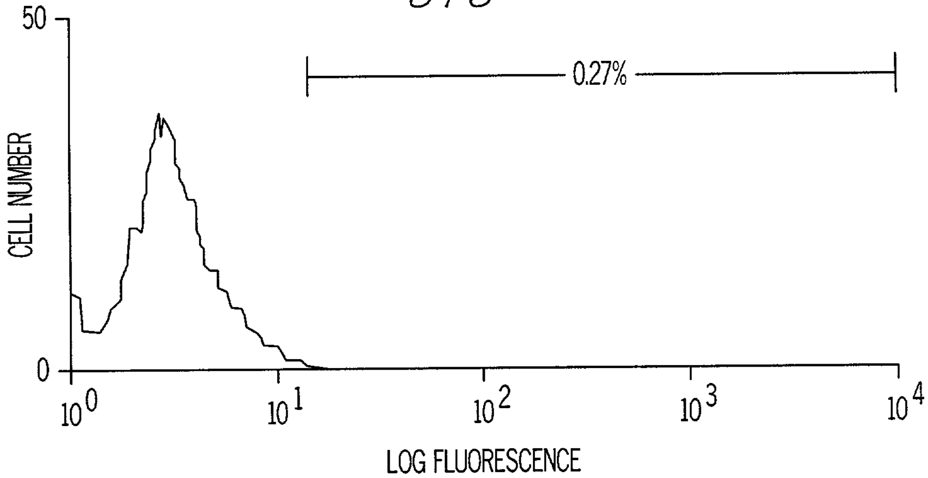

FIG. 4A

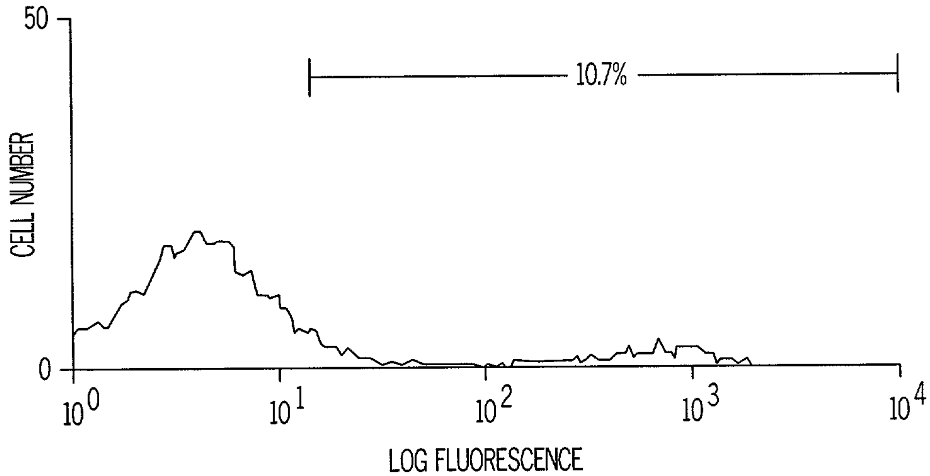

FIG. 4B

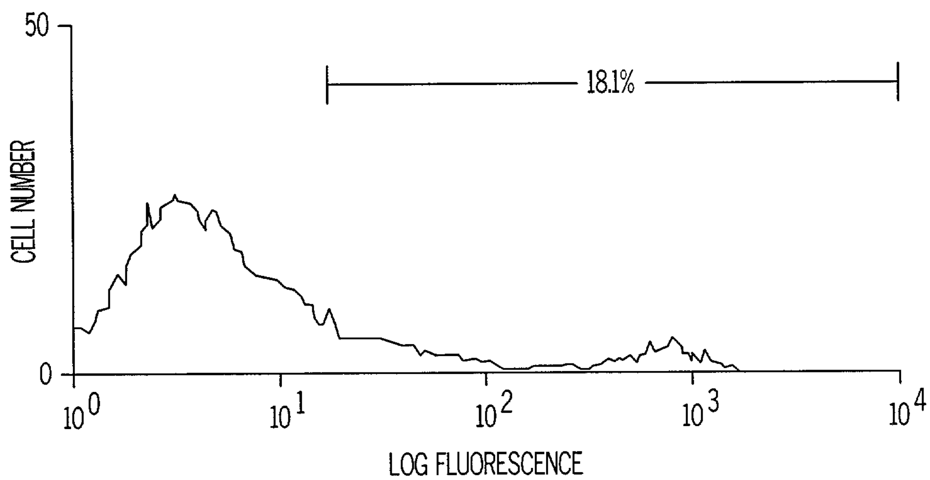

FIG. 4C